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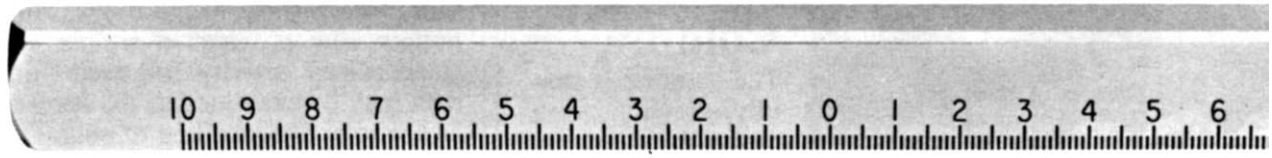

Analyzing Behavior-Related Neural Activity Via Correlation of Distribution Shape: Part 2

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INTRODUCTION

In Part 1 of this article, published in the last issue of the *Carrier,* we described the background of the rabbit nictitating membrane classical conditioning preparation, as initially described by Gormezano and his associates (Gormezano, 1962, Steinmetz & Thompson, 1991), including its basic parameters and the neural underpinnings of the conditioned response (e.g., Thompson, 1988). The classical conditioning paradigm used in this laboratory is shown in Figure 1 (page 4). We use a delay paradigm, with a 250 msec. preCS interval occurring immediatly prior to the onset of the 350 msec. CS. The CS is coterminous with a 100 msec. UCS. The data recording occurs during the three 250 msec. preCS, CS and UCS periods. The methods of relating neural activity to the behavioral data are presented here (see, e.g., Gould, Sears, & Steinmetz, 1991).

Standard scores of firing across time

The first step toward statistical analysis of the relationship between eyeblink and neural activity involves converting the raw number of action potentials into standard scores. To standardize the amount of neural firing, we first aggregate a block of 10 trials, summing the neural activities. Assuming that the neural pattern of activity is reasonably stable, the result is a 'peri-stimuli1 time histogram with a ten-trial bulge of activity around the border between the CS and US periods. We then divide each section of the aggregate block (pre-CS, CS, and US periods) into eight bins of the same length, each bin containing the number of action potentials produced within that particular time window (see note 1). With an equal number of bins in each period of the trial, we can compare stimulus- and response-related neural activity to the block-specific baseline. Each of the eight CS period bins and US period bins, numbered one to eight from the beginning to the end of the period, is compared to the same number bin of the pre-CS period.

Figure 2a (page 5) illustrates this technique, showing as an example the comparison between CS period bin eight and the corresponding baseline bin (pre-CS period bin eight). A simple t-test is performed on the average numbers of action potentials for the two bins. As the two averages are calculated from the same rabbit and the same trials, they are not considered to be independent of one another; therefore, a paired samples t-test is the appropriate measure (Hays, 1988). The results of the t-test is the standard scores for CS bin eight. If the average number of action potentials produced in CS bin eight is significantly higher than the average number of action potentials produced in pre-CS bin eight, then the standard score for that comparison will exceed the critical t-vaiue for the degrees of freedom of the t-test. If so, we can be reasonably confident that task-related neural activity appeared shortly before the onset of the US.

Figure 2b displays the output of this technique for a session involving twelve blocks of training.A matrix of 192 standard scores is computed, representing the level of task-related activity (across eight CS period bins and eight US period bins) for each of twelve blocks. In addition, the

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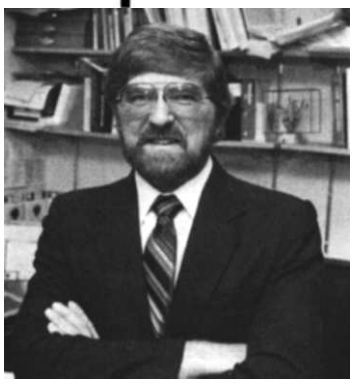

Editor's Column

The summer is rapidly drawing to a close. It seems to have gone by so fast. Perhaps for me and my wife, this is because we spent a part of the summer in St. Petersburg, Russia. I had been invited over there by a Russian physician/professor who was interested in learning more about osteopathic theory and manipulative treatment. I invited an osteopathic physician from Texas and his wife to go with us. We spent 17 days in St. Petersburg, teaching and being shown the wonders of one of the world's great cities.

We were there from June 17-July 3 which is the time of the "white nights." Since St. Petersburg is so far north, it simply does not get fully dark during the weeks around June 22. We could read the paper at 1 am! There was a dusk time about 2 am, but then the sun simply came up again. The residents of the city celebrate these long days, I suppose because they have only a few hours of daylight during the winter months.

Our time was spent teaching and seeing various things in the city and surrounding countryside. We taught in the professor's clinic the first week then on a cruise ship during the second week. Our hosts made sure we saw many of the famous places in city, including the Hermitage, the Peterhof and so forth. A good friend of the professor's, a Russian Army General treated us~ to a drive in a Russian Army tank (yes, I actually got to drive it), and the trip on the cruise ship up Lake Ladoga was spectacular.

We lived with and got to know a group of people who we used to think of as enemies. It was a profound experience in many ways, but primarily because it forever changed my view of another group of people. As I pointed out in my last column here, "getting to know someone is the best way to find out they are people, not objects."

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bottom row provides a simple average of the standardized activity for each bin, so that the data may be examined at the level of the session as a whole. The swelling of neural activity apparent in the histogram has now been quantified such that its statistical significance is made plain.

It might be argued that the sheer number of comparisons represented by the matrix drive the protection level-the probability of finding a seemingly significant result by chance alone-too high. While this may be true for an examination in which the mere presence of some significant results is the aim, it does not present much of a problem when the search is for a consistent pattern of increased firing block after block. In fact, by using session averages in the analysis, we tend to err on the side of caution. We require that the overall standard scores meet the more stringent critical values used for single blocks (in our case, df = 9).

This test is neither perfect nor adequate for our purposes, however. For one thing, background activity in the neural signal makes it difficult to detect brain-behavior relationships involving the inhibition of action potentials. The floor of noise may make an inhibition effect virtually undetect-able, even if it is the case that the in-task inhibition suppresses action potentials completely. Aggregating the data across several sessions will minimize this problem, but only if great care is taken to ensure that the baseline activity remains fairly constant for all such sessions. Such aggregation, meanwhile, rests on the perhaps invalid assumption that the sought-after inhibition is 4airly stable across sessions.

In fact, the baseline activity itself may not be stable, but may change with training. The stability of the baseline can be tested within a session, as long as amplifier gains are left untouched, by comparing the raw numbers of discriminated spikes from the first and last blocks. Between sessions, the validity of baseline activity comparisons is difficult to support.

Another limitation of the standardization procedure is that it only tells us whether the neural activity exceeds baseline; that is, the t-tests tell us whether the height of the histogram is greater than zero at each time point. They tell us nothing about the relationship between adjacent bins, or about the distribution as a whole. The shape of the distribution remains mysterious, as does its relationship, in shape and timing, to the eyeblink.

Cross-correlation analysis with behavior

To deepen the analysis, we directly compare the distribution of standardized neural activity across time to the distribution

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of eyeblink activity across time. The data are arranged such that the height of the standardized neural distribution at each time point is paired with the height of the behavioral distribution at the same time point. With the data set arranged in this way, the two distributions can be cross-correlated: a Pearson product-moment correlation (Hays, 1988) of the paired time points allows us to discern the similarity between the two distributions throughout the task. The obtained r-score provides a good estimate of how similar the shapes of the distributions truly are. Obviously, significance tests may be brought to bear on this r-score.

More than this is required, however, to relate a distribution of neural activity to the shape of a behavior that is presumably caused by the neural activity. The transmission of neural commands to effectors takes time-a signal must travel down axons, navigate synapses, and induce muscle activity. It is necessary to take this delay into account when cross-correlating the two distributions. For that reason, we 'slide1 the two distributions past each other, offsetting them by six msec at a time. At each offset, we calculate a new cross-correlation on the overlapping portions of the distributions.

The value of calculating cross-correlations at various offsets is potentially large. Fisher's z-tests (Hays, 1988) reveal how significantly each correlation differs from zero. The highest z-score tells us, at long last, what the best-fitting relationship between behavior and brain is. Perhaps more importantly, the peak z-score also provides information about the time lag between neural activity and behavior (see note 2). This latter measure, and its reliability across blocks, can be used as converging evidence that the recorded activity is neural activity (as opposed to artifactual noise caused by the movement) that is tightly linked to the behavior. For instance, conditioned eyeblinks and action potentials recorded from the interpositus nucleus tend to correlate as highly as r = .80 and .90. These high correlations cluster at an offset of approximately 30 msec, a biologically plausible delay given the synapses and distance between the interpositus and eye muscles. Taken together, these data have strengthened our argument that the interpositus is involved in the execution of a conditioned eyeblink. Interpositus activity does not reflect stimulus- or response-driven activity, nor does it merely signal response onset. It is, instead, involved in the production of CRs.Of course, time-lagged cross-correlation is not without its drawbacks. The largest concern can be summarized in a single word: gain. Cross-correlation assumes that changes in the two distributions are linearly related. That is, it assumes that a change of size x in the height of the neural activity histogram will always be associated with a change of size y in eyeblink size. If, for instance, arithmetic increases in the number of spikes cause multiplicative changes in the size of a blink, then the resultant cross-correlation will underestimate the relationship between the two. The fact that the records (neural and behavioral) are collected by separate amplification systems compounds the problem.

Another potential limitation of the procedure has to do with the possible imprecision of the 'best' correlation judgment. As with the spike standardization t-tests, the Fisher's z-tests compare the observed r-scores to an r-score of zero; they do not compare the observed r-scores at similar offsets. It is possible that the correlations observed at adjacent time lags are not significantly different from each other. In essence, it is possible that a large confidence interval exists around the 'best offset.1 As with the spike standardization procedure, however, this problem is minimal given the phenomenon observed in our preparation. If the most significant correlation is found at similar offsets for two sessions (24 blocks) in a row, it is reasonable to ignore the confidence interval of each individual calculation.

Summary

The power of the spike standardization and cross-correlation procedures lies in their ability to provide more fine-grained, qualitative analyses of the relationship between neural activity and individual behaviors. This paper has described these procedures as they pertain to rabbit conditioned eyeblink, but the detail should be sufficient for other researchers to institute them as well.

Notes

1. The number of trials in a block, and the number of bins in a period, are up to the discretion of the experimenter. We feel that ten trials per block, and eight bins per period, provide an optimal compromise between the need to keep the error level of the statistical comparisons low and the need to keep the temporal grain of the analysis fine.

2. Note that it is important, when hunting for the 'best' offset time between the distributions, to look at the significance of the correlations in addition to their size. At larger offsets, fewer time points will be entered into the analysis. Correlations based on fewer points will tend to be larger than those based on many points. Since significance depends on sample size (df), however, these larger correlations may not be more significant.

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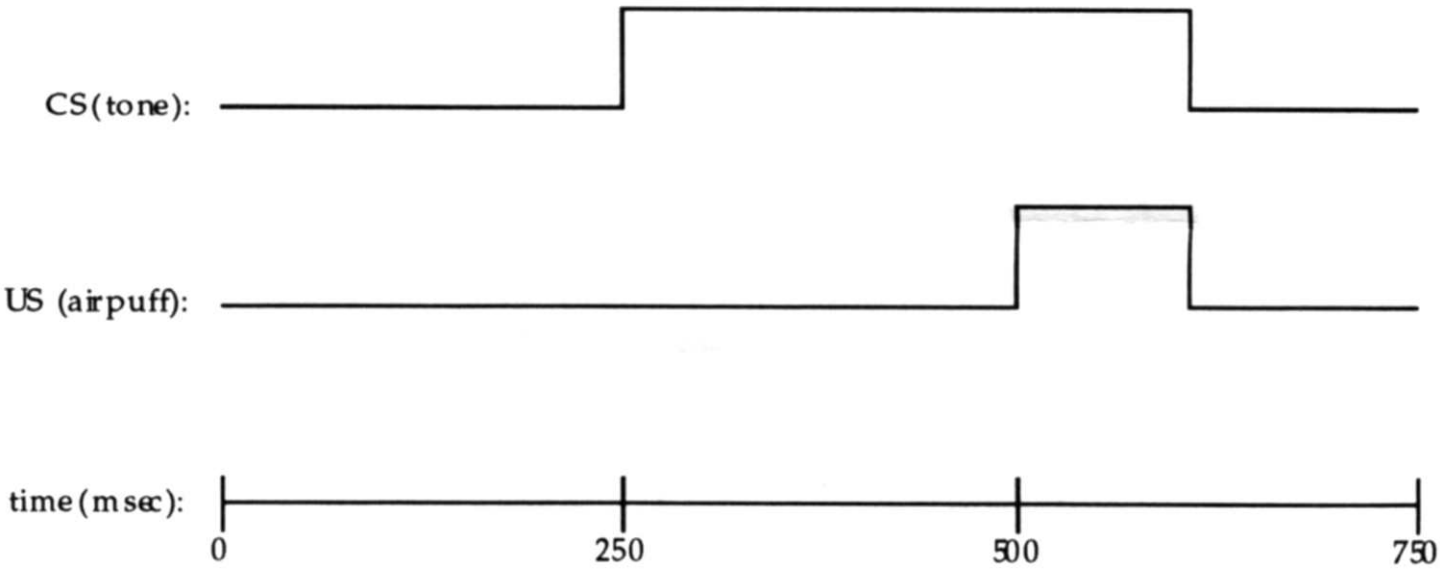

Figure 1. Illustration of the standard paired trial for rabbit eyeblink conditioning. The top line represents the CS and the bottom line represents the US; when a line is at its higher plateau, the stimulus is present. Time is represented along the horizontal axis of the figure.

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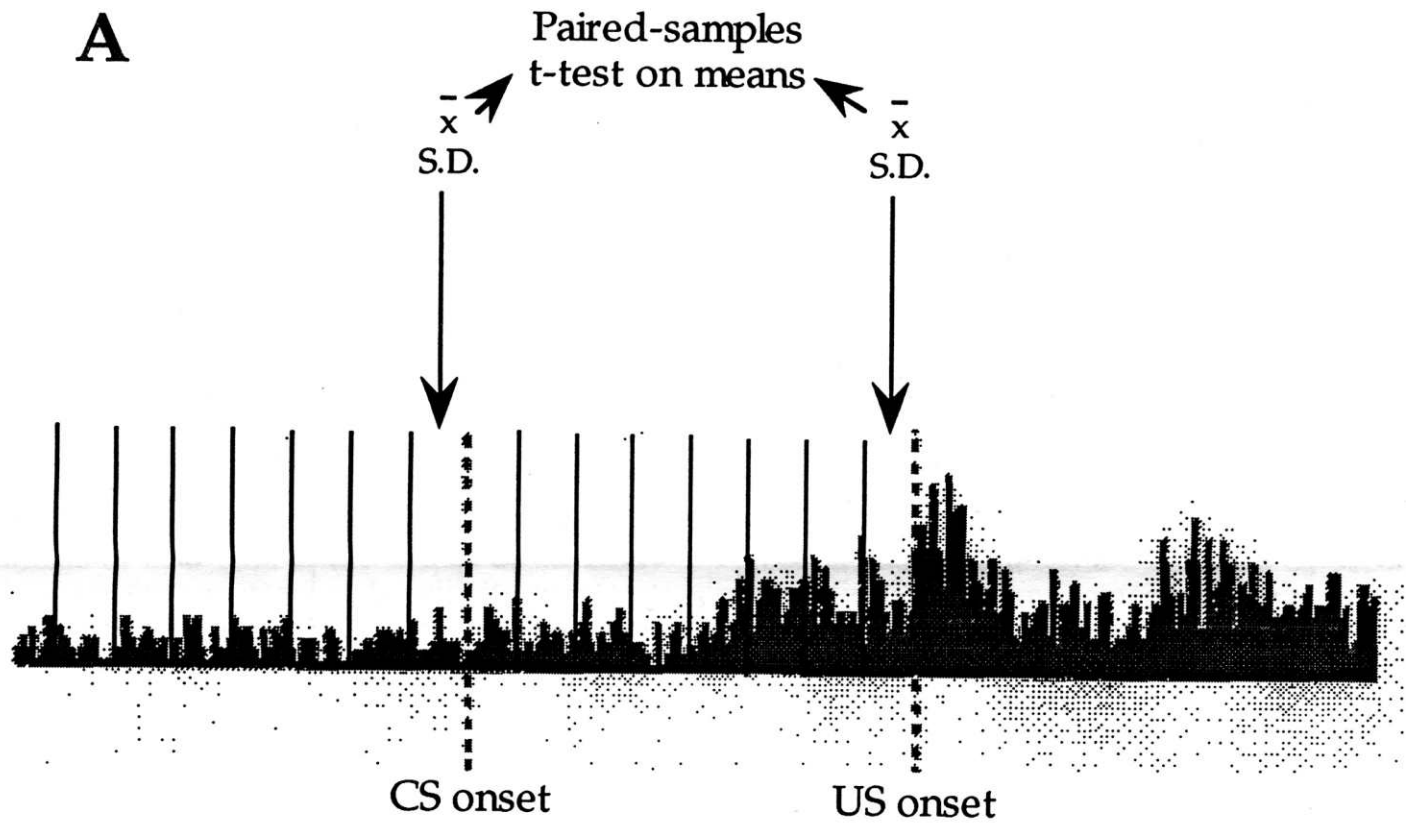

B

	cs1	cs2	cs3	cs4	cs5	cs6	cs7	cs8	us1	us2	us3	us4	us5	us6	us7	us8
1	4.12	X	3.14	3.54	6.55	5.71	6.79	6.71	7.21	5.75	4.39	2.86	4.04	X	3.10	X
2	X	X	2.97	2.33	X	2.41	7.06	4.11	6.47	2.22	X	X	X	1.86	X	X
3	X	X	2.76	2.49	4.02	4.04	4.94	2.58	5.08	2.34	X	X	X	X	X	X
4	2.70	X	2.34	3.50	3.15	X	2.74	5.19	4.77	X	X	X	X	X	X	X
5	X	3.28	X	2.43	X	X	X	3.12	7.58	7.80	X	2.41	X	X	-2.78	-1.93
6	X	2.53	X	2.71	4.95	5.85	5.25	9.00	4.78	3.63	X	X	X	X	X	X
7	2.38	X	2.43	3.24	3.60	3.64	4.95	4.26	5.49	X	X	2.24	X	X	X	X
8	X	X	X	5.41	X	4.02	5.76	5.45	6.97	X	X	2.04	X	2.65	X	X
9	2.45	4.02	X	X	3.07	7.52	4.09	3.63	7.96	4.62	3.15	3.19	5.32	6.25	6.62	4.71
10	X	1.94	2.73	3.35	3.52	X	3.45	3.43	5.17	3.71	X	X	X	X	X	X
11	X	6.82	X	X	X	4.87	3.61	6.98	4.65	3.55	X	X	X	2.25	X	3.05
12	2.21	3.84	1.89	2.47	3.04	9.13	3.37	3.23	7.06	5.30	3.04	X	X	X	X	X
	1.74	2.44	1.87	2.67	2.95	4.11	4.41	4.81	6.10	3.38	1.55	1.68	1.24	1.40	1.10	0.48

Figure 2. Analysis of the neural activity presented in Part 1. A. The last of eight CS-period blocks is related to the last of eight baseline pre-CS period blocks. A paired-samples t-test compares the difference between the two, using the means and standard deviations of the spike counts. B. The entire session's data presented in a matrix of t-values; block 9 corresponds to the above histogram. Columns are the eight CS-period bins and eight US-period bins. Rows are consecutive blocks. At the bottom are the session averages. The critical t-value is approximately 1.8; matrix spaces marked with 'x1 showed no significant activity.